

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY ONLY TEMPLATE**

**A. 510(k) Number:**

k120497

**B. Purpose for Submission:**

New device

**C. Measurand:**

Glycosylated Hemoglobin (HbA1c)

**D. Type of Test:**

Quantitative, immunoturbidimetric assay

**E. Applicant:**

Medica Corporation

**F. Proprietary and Established Names:**

EasyRA HbA1c Reagent  
EasyCAL HbA1c Calibrator  
Easy QC HbA1c QC Materials

**G. Regulatory Information:**

1. Regulation section:

21CFR 864.7470  
21CFR 862.1150  
21CFR 862.1660

2. Classification:

Class II for EasyRA HbA1c Reagent and EasyCAL HbA1c Calibrator;  
Class I, reserved for EasyQC HbA1c QC materials

3. Product code:

LCP – Assay, glycosylated hemoglobin

JIT – Calibrator, secondary  
JJX – Single, analyte controls

4. Panel:

Hematology (81), Chemistry (75)

**H. Intended Use:**

1. Intended use(s):

See Indications for use below

2. Indication(s) for use:

EasyRA HbA1c Reagent kit is intended for use in the quantitative in-vitro diagnostic determination of hemoglobin A1c (HbA1c) in human whole blood using the EasyRA clinical chemistry analyzer. HbA1c measurements are used for the monitoring of long term blood glucose control in diabetic patients.

The EasyCAL HbA1c calibrator is used for calibrating the HbA1c on the EasyRA clinical chemistry analyzer when used in conjunction with EasyRA HbA1c Reagent. The EasyCAL HbA1c calibrator is used to establish points of reference that are used in the determination of values in the measurement of HbA1c in human whole blood.

The EasyQC HbA1c QC Materials are intended to use as quality control material for the HbA1c immunoturbidimetric assay, using EasyRA HbA1c Reagent and EasyCAL HbA1c calibrator on the EasyRA clinical chemistry analyzer.

3. Special conditions for use statement(s):

For prescription use only

In the package insert the manufacturer has stated the following limitations:

False low values (low HbA1c despite high blood glucose) may occur in people with conditions with shortened red blood cell survival (hemolytic diseases) or significant recent blood loss (higher fraction of young erythrocytes). False high values (high HbA1c despite normal blood glucose) have been reported in iron deficiency anemia (high proportion of old erythrocytes). These circumstances have to be considered in clinical interpretation of HbA1c values.

Since significance interferences are found with Hb variants C, S and F; the sponsor has stated the following limitations in the package insert:

EasyRA HbA1c assay is subject to positive interference from HbC and HbS , and negative interference from HbF.

The sponsor has the following limitation in their labeling: “Patients with severe

gammopathy will generate erroneous result. Do not use this assay on patients with severe gammopathy.”

4. Special instrument requirements:

EasyRA Chemistry Analyzer

**I. Device Description:**

The EasyRA HbA1c Reagent kit is ready to use *in vitro* diagnostic reagent supplied in two wedges. Each wedge contains the following three reagent bottles:

R1 reagent (30 ml): Buffer Reagent, 20 mmol/L; Latex, 1.5 %

R2 reagent (10 ml): Buffer Reagent, 10 mmol/L; Mouse anti-human HbA1c monoclonal antibody, 5.5 mg/dL

R3 reagent (7 ml): Buffer Reagent, 10 mmol/L; Goat anti-mouse IgG polyclonal antibody 67 mg/dL; Stabilizers

The EasyCAL HbA1c calibrator set contains 4 vials of different levels of liquid-stable HbA1c calibrators. Each level is 0.25 mL.

The EasyQC HbA1c QC Materials contains 6 vials of different levels of quality control material for human HbA1c. Each level is 0.25 mL.

Hemolyzing reagent is provided in a liquid, ready-to-use bottle containing 125 mL of hemolyzing solution (DI water and 0.1% preservative).

All human source materials were tested by FDA approved methods and found to be negative for the presence of HBsAg and antibody to HIV1/HIV2, and HCV.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

Pointe Scientific HbA1c Reagent  
Pointe Scientific HbA1c Calibrator Set  
Pointe Scientific HbA1c Control Set

2. Predicate 510(k) number(s):

k031539

3. Comparison with predicate:

Similarities and Differences : Reagent		
Item	Predicate Device (k031539)	Candidate Device (k120497)

Intended use/Indications for use	For quantitative determination of hemoglobin A1c in whole blood. It is intended for health care providers in the monitoring of long-term glycemic control in individuals with diabetes mellitus.	Same
Instrument	Automated Hitachi 717	Medica EasyRA Chemistry Analyzer
Method	Latex agglutination, immunoturbidimetric assay	Same
Reporting units	% HbA1c	Same
Sample type	Whole blood hemolyzed	Same
Calibration frequency	With each run	7 days
Traceability	Traceable to NGSP, Diabetes Control and Complications Trial (DCCT) method	Same

Similarities and Differences : Calibrator		
Item	Predicate Device (k031539)	Candidate Device (k120497)
Intended use/Indications for use	For calibrating the HbA1c on the analyzer when used in conjunction with the HbA1c Reagent.	Same
Storage Temperature	2 ~ 8 °C	Same
Method	The calibrator is used to establish the calibration curve, which determines the HbA1c % in the patient samples.	Same
Hemolyzed before use	No	Yes

Similarities and Differences : Calibrator		
Item	Predicate Device (k031539)	Candidate Device (k120497)
Intended use/Indications for use	For monitoring the precision and accuracy of the HbA1c assay	Same
Storage Temperature	2 ~ 8 °C	Same

Hemolyzed before use	No	Yes
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**K. Standard/Guidance Document Referenced (if applicable):**

1. CLSI Guideline, EP5-A2 *Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline Second edition*
2. CLSI Guideline, EP7-A2 *Interference Testing in Clinical Chemistry; Approved Guideline- Second edition*
3. CLSI Guideline, EP6-A *Evaluation of the Linearity of Quantitative Analytical Methods; Approved Guideline*
4. CLSI Guideline, EP17-A *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline*
5. CLSI Guideline, EP9-A2 *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline Second edition*
6. CLSI Guideline, EP10-A2 *Preliminary evaluation of quantitative clinical laboratory methods*

**L. Test Principle:**

The EasyRA HbA1c assay employs the principles of immunoturbidimetry. Pre-determined amounts of patient whole blood are mixed with reagents R1, R2, and R3. Total Hb and HbA1c in hemolyzed blood bind with the same affinity to latex particles in R1. Mouse anti-human HbA1c monoclonal antibody (R2) binds to particle bound HbA1c. Goat anti-mouse IgG polyclonal antibody (R3) interacts with the monoclonal mouse anti-human HbA1c antibody and agglutination takes place. The measured absorbance is proportional to the HbA1c bound to latex particles, which in turn is proportional to the percentage of HbA1c in the sample. The turbidity is measured at 600 nm, and the percentage of HbA1c in unknown samples is derived from a calibration curve established by the HbA1c Multi-Calibrator kit using the Logit/Log4 curve fitting routine. The result is reported as the %HbA1c.

Sample preparation step: All samples, calibrators, and controls must be hemolyzed by mixing 20uL of the sample with 1000uL of the hemolyzing solution. The hemolysates must be mixed thoroughly and stand for 5 minutes or until complete lysis is apparent before use.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

Two levels of HbA1c EasyQC whole blood-based Quality Control material (Level 1 & 2) were tested. A third level of QC material was prepared by combining the two levels 1:1.

Within-Run and Total precision were determined following CLSI EP5-A2. Each QC level was hemolyzed and analyzed in duplicate on one EasyRA analyzer twice a day (AM and PM) over a twenty-day period. The data are summarized below.

Total sample numbers: 80 for each level

	Level 1	Level 2	Level 3
Mean %HbA1c	6.02	11.53	9.32
Repeatability SD, (Sr)	0.14	0.21	0.19
Repeatability %CV = (Sr/mean)*100	2.31	1.86	2.01
Total precision SD, (ST)	0.24	0.33	0.27
Total precision %CV = (ST/mean)*100	3.94	2.89	2.94

A second precision study was performed using two whole blood pools, one with %HbA1c values at 6.5 -7.0% and the other (spiked) at 11-14% respectively. Each pool was run 20 times within 4 hours after the EDTA whole blood samples were draw.

N=20 for each level	Level 1	Level 2
Mean %HbA1c	6.98	13.22
Repeatability SD, (Sr)	0.16	0.32
Repeatability %CV = (Sr/mean)*100	2.25	2.45

*b. Linearity/assay reportable range:*

The claimed linearity range is 4-14% for HbA1c.

The linearity of the HbA1c test was evaluated according to the CLSI EP6-A guideline using a commercially available Glycohemoglobin A1c linearity material. The linearity study used a high and a low patient pool to create additional intermediate levels of samples. A total of 7 samples with HbA1c concentrations ranging from 3.92% to 16.26% were tested in duplicate on a Medica EasyRA Analyzer. A polynomial regression analysis was performed and the sponsor determined that the 3<sup>rd</sup> order regression provided the best fit. However, the % difference between the 1<sup>st</sup> order regression and the best fit 3<sup>rd</sup> order regression is less than 0.75% (A1c units) at all linearity levels tested as summarized in the table below:

**Comparison of 1<sup>st</sup> Order and 3<sup>rd</sup> Order (best fit) regressions**

Expected Value (%)	1st Order value (%)	3rd Order value (%)	1st – 3rd Difference (%)
3.92	3.73	3.57	-0.15
5.87	5.66	5.89	0.23
7.84	7.61	7.81	0.20
9.98	9.74	9.67	-0.07
12.15	11.88	11.57	-0.31

14.19	13.91	13.66	-0.25
16.26	15.96	16.30	0.34

The 1<sup>st</sup> order linear regression generated is:

$$Y = 0.9911X - 0.1587, r^2 = 0.9958$$

The results of the study support the sponsor's claim that the assay is linear from 4 to 14% HbA1c.

*c. Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability:

The EasyRA HbA1c assay is traceable to the Diabetes Control and Complications Trial (DCCT) Method for Measurement of HbA1c. HbA1c values are reported according to the National Glycohemoglobin Standardization Program (NGSP) recommendations at the DCCT level.

An NGSP certification was provided with an expiration date of June 1, 2012. NGSP certification expires one year from the certification date and needs to be renewed annually.

Value Assignment:

Calibrator and control values are assigned by multiple measurements using multiple EasyRA analyzers and IFCC traceable calibrators.

Stability:

The stability protocols and acceptance criteria were reviewed and determined to be adequate.

The EasyRA HbA1c reagent, calibrators and quality control material are stable for 12 months at 2 – 8 °C; the expiration date is shown on the product label. The EasyRA HbA1c reagent is stable on-board for 30 days. The opened calibrators are stable for 30 days at 2 – 8 °C. The QC materials are stable for 3 months at 2 – 8 °C.

*d. Detection limit:*

A detection limit study was performed by the sponsor. LOB was determined using a blank sample, LOD was determined using a whole blood sample prepared with an HbA1c value slightly above the LOB. Each sample was analyzed 20 times on three EasyRA analyzers. LOB was calculated to be 0.815% and LOD was calculated to be 1.24%

The sponsor's claimed measuring range is 4% to 14% HbA1c.

*e. Analytical specificity:*

- i.) Studies were performed to assess common or known substances that could interfere with the EasyRA HbA1c assay. The interfering substances were evaluated in whole blood EDTA samples that contained three different concentrations of A1c (~5%, ~8% and ~12%). Samples containing various concentrations of potential interferents were tested and the results compared to those obtained from control samples containing no potential interfering substances. The sponsor's definition of non-significant interference is  $\leq 10\%$  difference between the tested and the control samples.

The percent difference between the control sample and the sample spiked with the potential interfering substances was no greater than  $\pm 10\%$  for concentrations at or below those listed in the following table.

<b>Potential interfering substance</b>	<b>Concentration at which no significant interference (<math>\leq 10\%</math>) was observed</b>
Ascorbic acid	$\leq 50$ mg/dL
Acetaldehyde	$\leq 100$ mg/dL
Acetylsalicylic acid	$\leq 100$ mg/dL
Bilirubin	$\leq 32.7$ mg/dL
Glybenclamide	$\leq 3$ mg/dL
Rheumatoid Factor	$\leq 960$ IU/ml
Sodium cyanate	$\leq 100$ mg/dL
Triglyceride	$\leq 2257$ mg/dL
Urea	$\leq 100$ mg/dL

- ii.) To study the interference from labile A1c, three EDTA whole blood patient samples with A1c concentrations approximately at ~5%, ~9% and ~13% were split into two aliquots. One aliquot of each concentration was supplemented by the addition of an aqueous glucose stock solution; up to 1500 mg/dL of glucose solution was tested. The samples were incubated for three hours at 37°C to facilitate formation of labile A1c. The samples were then tested on the EasyRA analyzer in duplicate. The sponsor's definition of non-significant interference is  $\leq 10\%$  difference between the spiked and non-spiked samples.

The sponsor concluded that labile A1c concentrations up to 1500 mg/dL do not interfere with the assay.

- iii.) To study the interference at multiple concentrations for variants S, C, D, E, and F, patient samples with known concentrations of variant were mixed together in different proportions with samples that did not have any variants. All samples were tested using the EasyRA analyzers and a commercially available HPCL method that was free of interference from the variants. The sponsor's definition of non-significant interference is  $\leq 10\%$  difference between the candidate method



and the comparative method.

The testing results shows there is positive interference for HbC and HbS; there is negative interference for HbF at 11.9%; and there is no significant interference for HbD up to 36.5%; HbE up to 30%.

Since significance interferences are found with Hb variants C, S and F; the sponsor has stated the following limitations in the package insert:

EasyRA HbA1c assay is subject to positive interference from HbC and HbS , negative interference from HbF, and no interference from HbD and HbE variants.

- iv.) To study the possible interference of gamma globulin in patients with monoclonal gammopathy, HbA1c testing was performed in triplicate on EasyRA with whole blood at two levels of HbA1c (6.5% and 11.5%) in the presence of various concentrations of gamma globulin. The HbA1c and corresponding Total Protein (as monitor of gamma globulin) values were determined, the % HbA1c recovery was calculated relative to the control. The sponsor's definition of non-significant interference is  $\leq 10\%$  difference between the spiked and the control sample. The results showed that, with HbA1c at 6.5%, there was no significant interference up to 3.3 g/dL of  $\gamma$ -Globulin (corresponding to Total Protein of 10.9 g/dL); with HbA1c at 11.4%, there was no significant interference to 6.6 g/dL of  $\gamma$ -Globulin (corresponding to Total Protein of 14.7 g/dL).  
The sponsor has the following limitation in their labeling: "Patients with severe gammopathy will generate erroneous result. Do not use this assay on patients with severe gammopathy."

*f. Assay cut-off:*

Not applicable.

## 2. Comparison studies:

*a. Method comparison with predicate device:*

Method comparison studies were performed according to the CLSI EP9-A2 guideline. 148 whole blood EDTA samples with HbA1c ranging from 4.2% to 13.7 % were analyzed in singlicate using EasyRA HbA1c assay on the EasyRA analyzer (candidate device) and the Pointe Scientific HbA1c Reagent on Roche Cobas-Mira analyzer (predicate device).

The linear regression correlation was calculated as follows:

$$Y = 1.0186X - 0.1104, r^2 = 0.986 \text{ (X=predicate method, Y=candidate method)}$$

95% CI of slope is 0.9961 to 1.0411

95% CI of intercept is -0.2778 to 0.0570

*b. Matrix comparison:*

Not applicable

3. Clinical studies:

*a. Clinical Sensitivity:*

Not applicable

*b. Clinical specificity:*

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

In the labeling the sponsor states that the HbA1c concentrations of an average whole blood specimen from a healthy individual should be less or equal to 6.0% (<92 mmol/mol).\*

\*Tietz Clinical Guide to Laboratory Tests, 4<sup>th</sup> edition, Saunders Elsevier, St. Louis, MO

**N. Proposed labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.